Potential anti-proliferative effects of chemical constituents and hemisynthetic derivatives from *Scadoxus pseudocaulus* (*Amarillydaceae*)

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Abstract

Background: Biological significance of *Amaryllidaceae* is well advocated from the literature. In Cameroon, plants from this family are routinely used for the cure of liver, cancer and cardiovascular diseases. To date, no scientific investigation corresponding to the anti-cancer activity of extracts and isolated compounds of *Scadoxus pseudocaulus* is available.

Objective: Current study is focused to elaborate the anti-proliferative effects of natural isolates (compounds 1-6, 9) and hemi-synthetic analogs (compounds 7-8) extracted from *S. pseudocaulu*.

Methods: Column chromatography of the ethyl acetate extract followed by purification of different fractions led to the isolation of seven compounds (1 – 6, 9). Esterification reaction of compound 6 was carried out using butyroyl chlorides and triethylamin to produce two derivatives (7 – 8). The cytotoxic activity was performed after staining of treated cells with florescent dye propidium iodide. Dead cells were detected using cytometer FL2 or FL3 channels/filters.

Results: Trans-derivative of narciclasine (a natural isolate from *S. pseudocaulus*), was found to be most potent among all tested compounds. Its effects were more significant on low malignant follicular lymphoma (DoHH2 cells) as compared to highly malignant (EBV infected) Burkitts lymphoma (Raji cells).

Conclusion: From our results, narciclasine appears to hold the potential of a lead molecule that can be used to bridge the therapeutic gaps in cancer research.

Keywords: *Scadoxus pseudocaulus*, *Amaryllidaceae*, 7-deoxy-trans-dihydronarciclasin, farrerol, derivatization, cytotoxic activity.
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Introduction

Scadoxus pseudocaulus (*I. Bjornstad* and *Friis*), a member of the *Amaryllidaceae*, is used in the West Region of Cameroon, for the treatment of liver, cancer and cardiovascular diseases. Previous study demonstrated the antimicrobial, antioxidant and anti-butyrylcholinesterase activities of compounds and extracts of *S. pseudocaulus*.[1] Apart from these biological activities, no conclusive scientific study is reported pertaining to its anti-cancer activity. It is known

that alkaloids of *Amaryllidaceae* family (likehe non-basic hydroxylated phenanthridones) possess high cytostatic activity.[2-6] Some other alkaloids like galanthamine, lycorine and narciclasine can only be synthesized in plants of Amaryllidaceae family.[7] Alkaloids from this family bear a number of biological activities including anti-microbial and anti-cancer.[6] 7-deoxy-trans-dihydronarciclasin or trans-dihydrolycoricidin (5) is isocarbostyrl alkaloid reported from *S. pseudocaulus* like narciclasine (also known as lycoricidinol) who is known for its effects on protein biosynthesis.[8] Narciclasine oil is effective in the treatment for uterine tumors. It also acts as a plant growth modulator.[9] Narciclasine's first bioactivity was observed in 1967, where it was shown to have strong mitosis blocking activity.[10] The reported biologically effects of narciclasine includes: anti-proliferative, antitumor/cytotoxic, acetylcholinesterase inhibitory, analgesic, hypotensive, antibacterial and antifungal.[11] Data from studies on HeLa cell line showed that narciclasine and other alkaloids like lycorine, hidydrolycorine, haemanthanime, pretazettine and pseudolycorine can inhibit growth and protein synthesis.[12] Not only narciclasine but also other alkaloid derivatives of *Amaryllidaceae* were reported to have anti-cancer effects.[13,14]

However, narciclasine's group is found to be most potent and effective against cell growth and protein synthesis.[6,15,16] Narciclasine is also found active on murine (p388 lymphoma) and human cancer cell lines (e.g. A549, NSCLC, PC3 and prostate). Narciclasine was also proposed as potential tool to cure apoptosis resistant metastasizing cancer cells.[17] A study conducted on a panel of 60 human cancer cell lines showed narciclasine's potential cytotoxic effects.[15] Another study reported anti-cancer effects of narciclasine on a variety of cancer cell lines, where fibroblasts were reported to be comparably resistant.[18] Sensitivity of cancer cells to narciclasine was also reported using HUVECs (endothelial) cells.[19] In a series of mechanistic studies, narciclasine was found to induce apoptosis driven cell death in cancer cells either mediated by the death receptors or mitochondria.[18] It was further confirmed using human promyeloic (HL-60) cells and human oral cavity squamous carcinoma (HSC-2 cells) that it can induce apoptosis even at nano-molar concentrations.[20] The above literature highlighted the significant biological effects of *Amaryllidaceae* family's natural isolates and hemi-synthetic derivatives and advocates further investigations to discover their anti-cancer tendencies. Therefore, the current study aimed at evaluating the antiproliferative activities of isolated compounds and hemisynthetic derivatives from the whole plant of *S. pseudocaulus*.

Materials and methods

Plant material

Whole plant of *Scadoxus pseudocaulus* was collected at Dschang, Menoua Division, West Region of Cameroon, in May 2013. The plant material was identified by Mr. Victor Nana, a botanist at the National Herbarium, Yaoundé, where a voucher specimen (N° 34986/SRF/CAM) was deposited.

Extraction, isolation and hemi-synthesis of compounds

The extraction and isolation of compounds were done as previously described.[1] Briefly, *S. pseudocaulus* was air-dried and powdered. The powder was macerated at room temperature with MeOH to afford the MeOH extract. The CHCl3 and EtOAc fractions from the MeOH extract were collected by column chromatography. Purification of the EtOAc fraction yielded seven known compounds (Figure 1). Esterification reaction of compound 6 was carried out using butyroyl chlorides and triethylamin to produce two derivatives. The structures of isolated compounds and derivatives were determined thrugh NMR and MS. The data thus obtained was compared with those from the literature.

Cytotoxicity assay

To assess the anti-cancer potential of natural isolates and hemi-synthetic analogs, Burkitt's and Follicular lymphoma (B lymphoma) cell lines were selected. These cell lines were a gift from Prof. Dr. Daniel Hoessli, Switzerland and from Dr A. Kluin-Nelemans, Groningen, Netherlands, respectively. Monkey Vero cells (African green monkey kidney cells, normal non-cancer cells, ATCC No. CCL-81), obtained from the American Type Culture Collection (ATCC), was also used in this study. The cell lines were maintained at 37 °C in a humidified 5% CO2 environment in Roswell Park Memorial Institute 1640 medium (RPMI; Caisson) with 1% L-glutamine, 1% penicillin/streptomycin (Gibco, Invitrogen), supplemented with 10% foetal bovine serum (FBS, PAA laboratories). The cytotoxicity analysis was performed after staining of treated cells with florescent dye propidium iodide (PI)

(excitation wavelength = 536 nm; emission wavelength = 617 nm).[21,22] Principally, PI can't cross the intact plasma membrane and therefore, cannot stain live cells. However, after the cells have lost membrane integrity (i.e. dead cells), this dye enters into the cells and intercalate with the cellular DNA. Dead cells thus fluoresce and can be detected using cytometer FL2 or FL3 channels/filters. To generate dose response curve of the set of standard and test compounds, cells were seeded in a 96 well plate. Each well contains 0.13x106 cells in final reaction volume of 200 µL. Cells treated with bosutinib + RPMI 1640 served as positive control whereas cells left untreated + 0.5% (v/v) DMSO + RPMI 1640 were used as negative control.

Statistical analysis

Data was analyzed by one-way analysis of variance followed by Waller-Duncan Post Hoc test and Statistical Package for Social Sciences software (SPSS, version 12.0). The results were expressed as mean ± standard deviation (SD). Differences between groups were considered significant when $p < 0.05$.

Results

Chemical analysis

The phytochemical investigation of the CHCl3 and EtOAc fractions from the MeOH extract of S. pseudocaulus, afforded six known compounds namely sideroxylin (1),23 5-hydroxylmethyl-2-furancarboxaldehyde (2),24 C-6,O-7-dimethyldenaromadendrin (3),25 4-(hydroxymethyl)-5-hydroxy-2H-pyran-2-one (4),26 7-deoxy-trans-dihydronarciclasin or trans-dihydrolycoricidin (5),15 farrerol (6)25,27 and pinoresinol-4'-O-β-D-glucopyranoside (9).28 Esterification reaction of farrerol (6) was carried out using butyroyl chlorides and triethylamin to produce two derivatives namely 4',7-butyroylfarrerol (7) and 4'-butyroylfarrerol (8)1 (Figure 1).

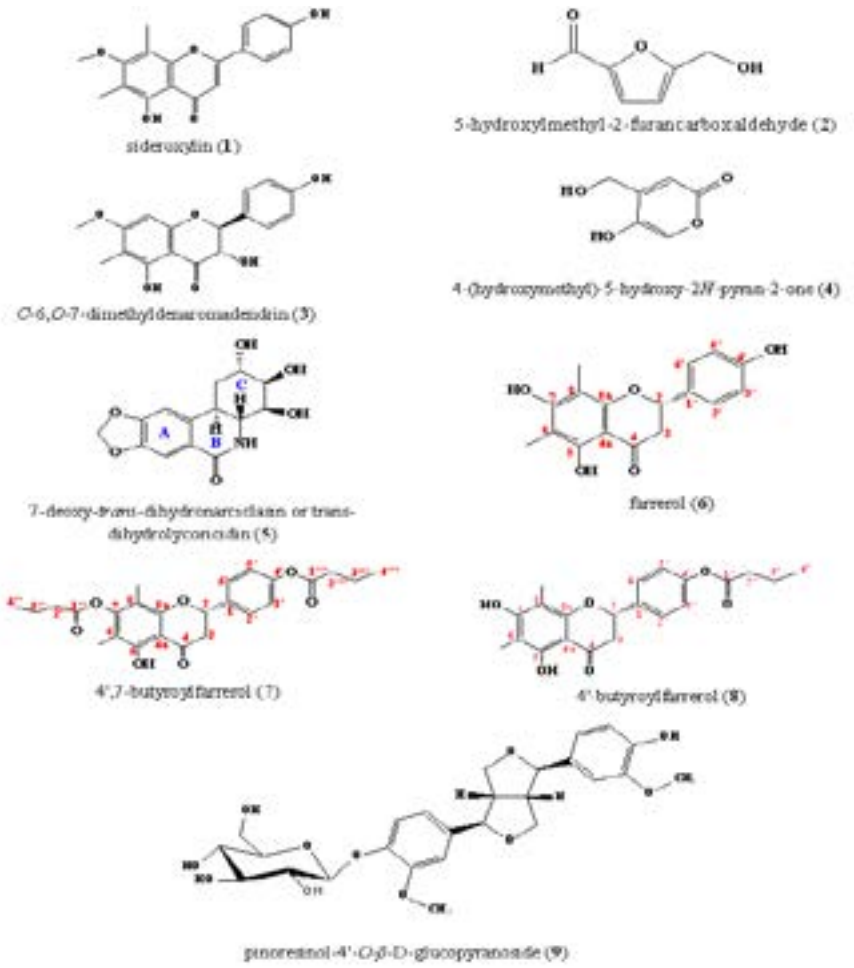

Figure 1: Structures of isolated compounds (**1-6, 9**) and hemisynthetic derivatives (**7-8**)

Cytotoxic activities

The cytotoxic activities of compounds[1-9] were evaluated against two cancer cell lines and normal non-cancer cells (Vero cells) (Table 1, Figures 2 – 3). Both cancer cell lines were found sensitive to all tested compounds (0.46 ± 0.18 to 46.15 ± 3.44), however, the highest activity was observed for compound 5 (-deoxy-trans-dihydronarciclasin or trans-dihydrolycoricidin) (46.15 ± 3.44 for Raji cells and 39.62 ± 1.67 for DOHH2 cells) (Figures 2 - 3). For Raji cells, 114.47 μM concentration of compound 5 was required for the induction of death in 50% cell population, however, 134.28 μM were found sufficient for DoHH2 cells (Table 1). The test compounds were non-toxic to normal cells (results not shown) whereas the Selectivity Index (SI) values of the compound 5 against the Raji and DOHH2 cells cells are 13.07 and 11.14 indicating its good selectivity on the test cancer cell lines (Table 1). Bosutinib was used as a standard drug in the study. Its IC50 dose for Raji cells observed to be 63.17 μM ± 3.65 and for DoHH2 cells 68.30 μM ± 1.06 after 48 hr incubation time (Table 1).

Table 1: Cytotoxicity (IC_{50}) and Selectivity index (SI) of compound **5** against Raji and DOHH2 cells.

Compounds	Cytotoxicity (IC_{50} in μM)			Selectivity index (SI)	
	Raji cells	DOHH2 cells	Vero cells	Raji cells	DOHH2 cells
5	114.47 ± 0.53[a]	134.28 ± 1.34[b]	1496.46 ± 1.84[c]	13.07 ± 1.71[a]	11.14± 0.98[a]
Bosutinib	63.17 ± 3.65[a]	68.30 ± 1.06[b]	1298.83 ± 1.09[c]	20.56 ± 1.54[a]	19.01 ± 1.23[a]

SI: IC_{50} on Vero cells/IC_{50} on cancer cells; *SI obtained from average IC_{50}; each IC_{50} value represents the Mean ± SD (n = 3); on the same line, IC_{50}/SI values marked with different supersript letters are significantly different ($p < 0.05$).

Discussion

The current investigation was carried out to evaluate the antiproliferative activities of isolated compounds and hemisynthetic derivatives from the whole plant of *S. pseudocaulus*. The findings of the present study revealed lesser sensitivity of Raji cells to compound 5 that can be due to EBV (Epstein Barr Virus) infection. This virus encodes a number of viral proteins that makes these cells resistant to apoptosis and promote growth.[29-31] One of these proteins is latent membrane protein (LMP2A) that is membrane bound and shares structural resemblance to B cell surface receptor protein (BCR). LMP2A is known to play role in relaying survival signal thus promoting survival of B lymphoma cells.[32-34]

7-deoxy-trans-dihydronarciclasin, (an enantiomer) is a well reported potent anti-neoplastic agent.[35] Trans derivative of narciclassine is found to be more active as compared to cis form.[13] 7-deoxy-trans-dihydronarciclasin has significant anti-cancer effects and the detailed SAR studies elucidated that this molecule has pharmacophore moiety which induces apoptosis.[15,19,36-40] The tri-hydroxylated ring C of this compound is also considered to be a critical part. In fact, the substitution of tri-hydroxylated ring C with hydrophobic moieties led to decrease in anti-cancer capacity and the same was observed upon loss of three hydroxyl groups.[37,41-44] Thus, it could be seen that 7-deoxy-trans-dihydronarciclasin has significant cytotoxic effects and can induce apoptosis in cancer cell lines making it a lead molecule in cancer research. The antiproliferative effects of sideroxylin against ovarian cancer cells are through the induction of mitochondrial dysfunction and the activation of PI3K and MAPK signal transduction.[45] Selectivity Index (SI) of active compounds was determined in order to investigate, whether the cytotoxic activity was specific to cancer cells. The SI of the samples is defined as the ratio of cytotoxicity (IC50 values) on normal cells (Vero cells) to cancer cells: SI = IC_{50} on Vero cells/IC50 on cancer cells. The Selectivity Index (SI) values of the compound 5 against the Raji and DOHH2 cells cells are 13.07 and 11.14 and could be considered as good when taking in consideration that the ratio for a good therapeutic index for a remedy or drug should be ≥10.[46] These results are consistent with the use of compound[5] for treating B lymphoma.

Conclusion

Present investigation has revealed that isolated compounds and hemisynthetic derivatives from *S. pseudocaulus* are active against the tested cancer cell lines and non toxic against Vero cells (non-cancer cells). The pattern of response revealed that EBV infected Burkitt lymphoma is less sensitive to 7-deoxy-trans-dihydronarciclasin as compared to Follicular lymphoma. Greater cytotoxic effect on slow growing follicular lymphoma signifies its metabolic stability which can be exploited for slow progressing malignancies. Further investigations are needed to screen them against other cancer types and human cell line of normal tissues, including bone marrow cells to justify the traditional use of *S. pseudocaulus* as an anticancer substance. Detailed mechanistic studies are also needed to find the mode of action of this compound on the molecular pathway(s) potentially leading to cell death.

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Conflict of interest

None declared.

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